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Total Synthesis of Cyclomarin A, a Marine Cycloheptapeptide with Anti-Tuberculosis and Anti-Malaria Activity

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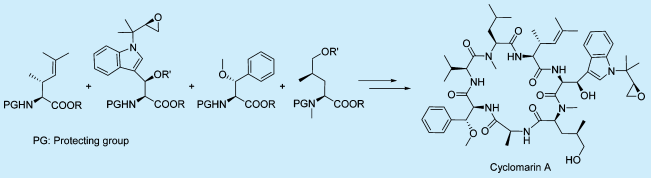
Supporting Information

ABSTRACT: An efficient synthetic protocol for the stereoselective synthesis of cyclomarin A is reported. Key steps in the syntheses of the building blocks are an asymmetric chelate–enolate Claisen rearrangement, an asymmetric hydrogenation, and highly diastereoselective additions of organozinc and -titanium reagents.



Most people in industrial nations believe that tuberculosis (TB) has been wiped out, but statistically almost every third human is infected by *Mycobacterium tuberculosis* (MTB).[1] Behind HIV/AIDS, TB stands at position two in the list of lethal infections.[2] Several multidrug resistant bacteria are resistant not only toward first-line medication but also toward second-line medicine.[3] Therefore, the development of new drugs, with a new mode of action, is highly desired.

In 1999, Clardy et al. described the isolation and structure elucidation of the cyclomarins from the marine streptomycete CNB-982 (Figure 1).[4] The crude extract showed a moderate

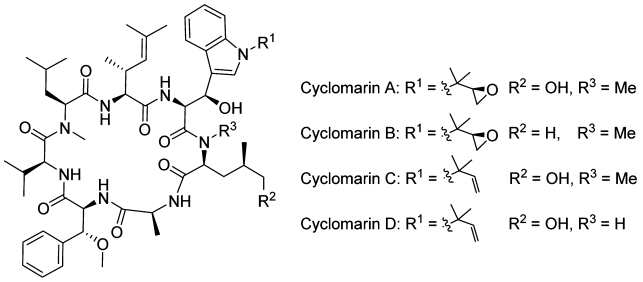


Figure 1. Natural occurring cyclomarins.

cytotoxicity, with an IC_{50} of ~2.5 μM of the major metabolite cyclomarin A (Cyc A) toward several cancer cell lines. The minor metabolites cyclomarin B and C (Cyc B and C) are only found in trace amounts (2–3%). Later, a further derivate cyclomarin D (Cyc D), missing an *N*-methyl group, was isolated from *Salinispora arenicola* CNS-205.[5] Besides the moderate cytotoxicity, the cyclomarins show some antiviral[6] and antibiotic activity, especially against MTB, while the exact mode of action was not clear until recently.[7] Detailed studies at Novartis identified ClpC1, a subunit of the caseinolytic protease, as the target protein. Very recently, the same researchers also reported significant antimalaria activity. Cyclomarin A selectively inhibits the $PfAp_3Ase$ of *Plasmodium falciparum* in a nanomolar range, but not the human homologue in FHIT.[8] To the best of our knowledge, a natural product with two different targets in two highly important human pathogens is unprecedented. To date, all studies have been carried out with cyclomarin A (and derivatives thereof) from fermentation, since no total synthesis has been reported. Only one synthesis of the minor metabolite cyclomarin C, lacking the epoxide functionality on the hydroxytryptophan, has been described by Yao et al.[9] Herein, we report the first total synthesis of cyclomarin A, which should open the way to synthetic derivatives for SAR studies to develop efficient drugs to fight both diseases.

Some of the unusual amino acids found in cyclomarins, e.g., 5-hydroxyleucine and the β-methoxyphenylalanine, are also present in other natural products.[10] Others, such as the *tert*-prenylated β-hydroxytryptophan and the adjacent 2-amino-3,5-dimethyl-4-hexenoic acid, are unique to cyclomarins. Although the synthesis of cyclomarin A has not been reported, several syntheses of building blocks have been described during the last several years.[11−14]

We developed our own synthetic protocols toward the required building blocks based on our long-term experience in amino acid synthesis.[15] When chelated amino acid ester enolates are used, β-hydroxy amino acids become easily available via an aldol reaction,[16] and γ,δ-unsaturated amino acids can be obtained either by transition-metal-catalyzed allylic alkylation[17] or chelate enolate Claisen rearrangement.[18] While allylic alkylation provides *anti*-configured products, the *syn* isomers can be obtained via Claisen rearrangement. Therefore, this approach was used here to generate the desired *N*-protected amino acid (2*S*,3*R*)-**4** (Scheme 1).

The chelate Claisen rearrangement of chiral ester (*S*)-**1** gave rise to the desired amino acid with perfect chirality transfer and diastereoselectivity.[18] After methylation, the double bond was cleaved via ozonolysis. Unfortunately, attempts to introduce the required terminal methyl group via Wittig reaction[9] failed. Even with a 5-fold excess of the Wittig reagent only 21% of the desired product **3** could be obtained, while the large excess of basic Wittig reagent caused complete epimerization of the β-methyl

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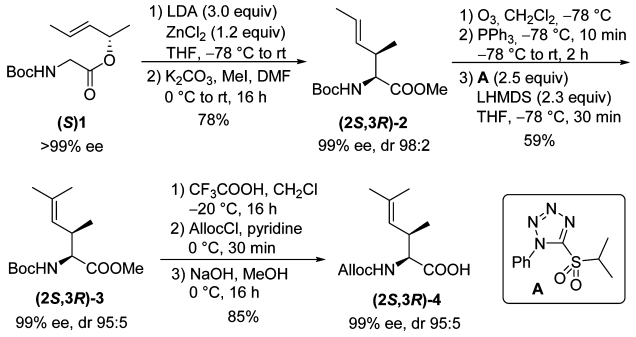
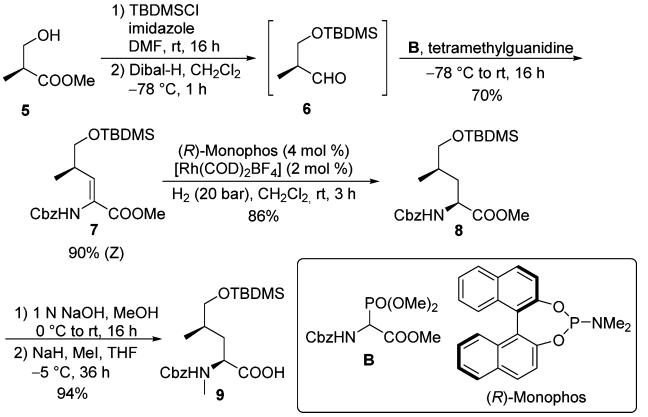
Scheme 1. Synthesis of (2*S*,3*R*)-4



group. Better results could be obtained using a modified Julia–Kocienski reaction,[19] normally used for (*E*)-selective olefinations. Sulfone **A** was deprotonated with LHMDS, and the solution was added to a fresh prepared aldehyde (without purification). Under these conditions, the protected amino acid **3** could be obtained without significant epimerization. During the synthesis, we recognized that the *N*-Boc-protecting group could not be removed in the peptide without several side reactions. Therefore, we replaced the Boc-protecting group by an Alloc group. Subsequent saponification gave rise to building block **4**.

The synthesis of protected 5-hydroxyleucine **9** (Scheme 2) started with the commercially available Roche ester **5**, which was

Scheme 2. Synthesis of (2*S*,4*R*)-9

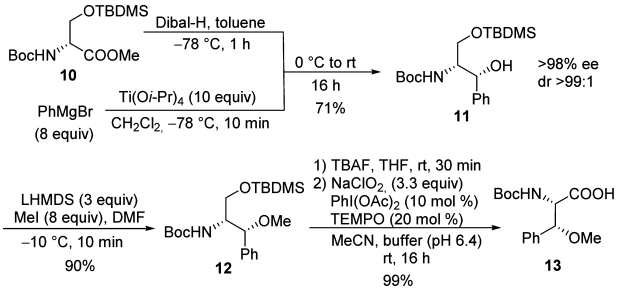


protected, reduced to the aldehyde **6**, and directly used in an olefination using Schmidt's phosphonoglycinate **B**.[20] The α,β-unsaturated amino acid derivative **7** obtained was subjected to a stereoselective hydrogenation[21] using (*R*)-monophos as a ligand.[22] Subsequent *N*-methylation and saponification provided amino acid **9** in high yield and selectivity.

The third unusual building block, β-methoxyphenylalanine **13**, should be accessible via chelate-controlled arylmetal addition toward a protected (*R*)-serinal, obtained from ester **10** (Scheme 3).[23] Our first attempts using phenylmagnesium bromide (3 equiv) gave the addition product in acceptable yield (60%) but only moderate diastereoselectivity (ratio 7:3). Much better results could be obtained with the corresponding titanium reagents,[24] providing **11** as a single diastereomer. *O*-Methylation, cleavage of the silyl ether, and subsequent oxidation gave rise to the desired acid **13** in excellent yield.

By far, most of our encounteded difficulties caused the synthesis of the *tert*-prenylated epoxidized β-hydroxytryptophan, mainly because of the lability of the β-OH group and the terminal

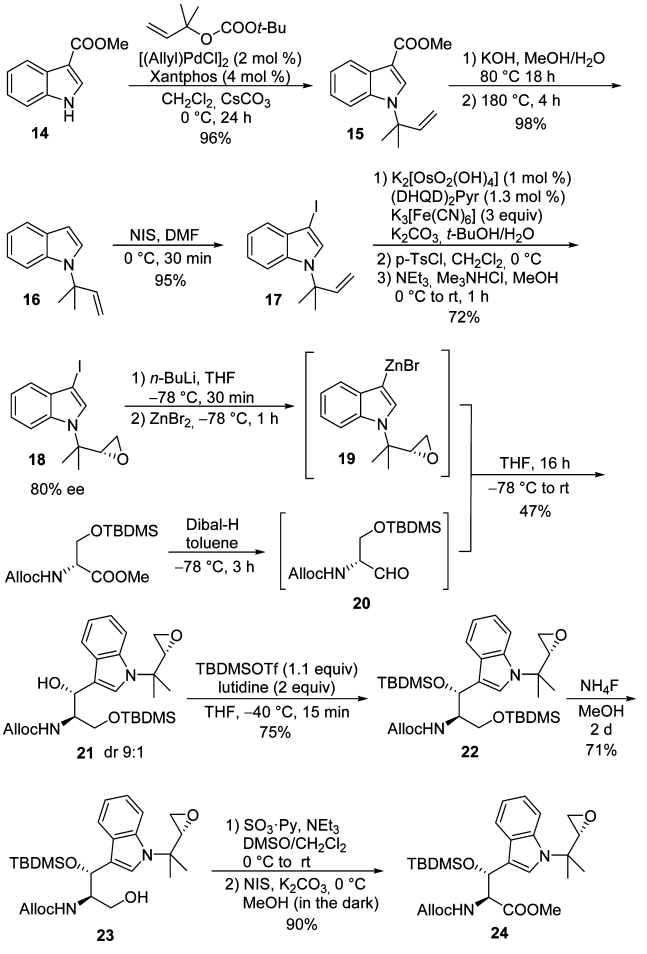
Scheme 3. Synthesis of (2*S*,3*R*)-13



double bond of the prenyl group. Recently, we reported the synthesis of an *N*-Boc-protected *tert*-prenylated β-hydroxytryptophan derivative,[25] which unfortunately could not be applied here, since the Boc-protecting group could not be removed without decomposition. Therefore, we had to develop a new protocol, introducing the epoxy group relatively early in the synthesis.

Recently, a new method was reported for the regioselective *tert*-prenylation of electron-demanding indoles.[26] We applied this Pd-catalyzed protocol to 3-indolecarboxylate **14**, giving rise to indole **15** in almost quantitative yield (Scheme 4). The reaction temperature was kept at 0 °C to suppress the competing *n*-prenylation. After saponification of the ester, the free acid was subjected to a thermal decarboxylation.[27] Heating the neat acid to 180 °C resulted in a clean decarboxylation in perfect yield. The

Scheme 4. Synthesis of (2*S*,3*R*)-25



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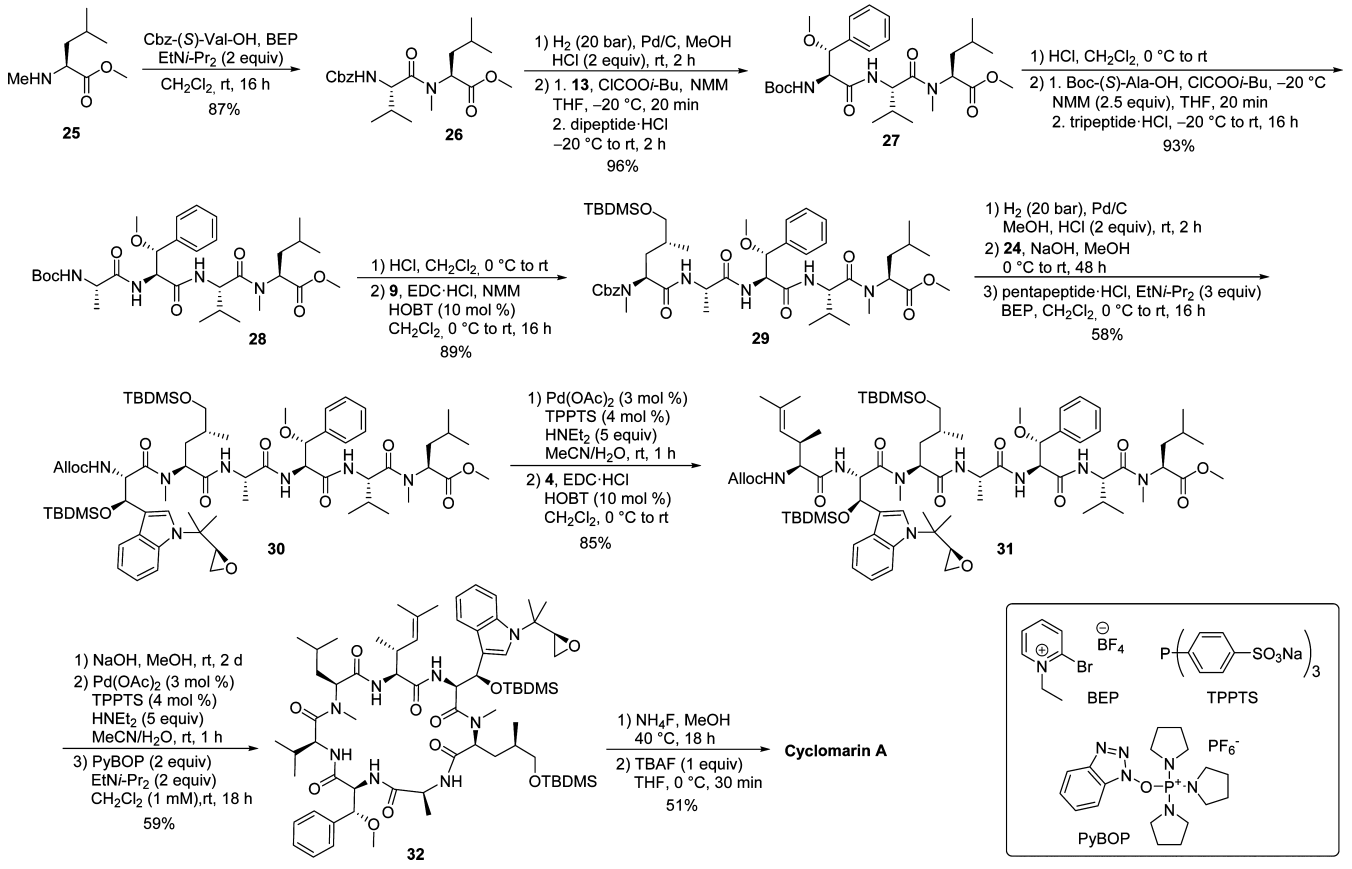
Scheme 5. Synthesis of Cyclomarin A



prenylated indole **16** was converted into the corresponding 3-iodo derivative **17**, and the terminal double bond was transformed into epoxide **18**[14b] using a Sharpless dihydroxylation as a stereodetermining step. Compound **18** was lithiated at −78 °C using BuLi and was subsequently transmetalated to the corresponding organozinc reagent **19**,[28] which was added to the solution of fresh prepared aldehyde **20**. The desired product **21** was formed in acceptable yield as a 9:1 diastereomeric mixture. Out of four possible stereoisomers, only two isomers were obtained and the isomeric mixture was the result of the moderate selectivity of the Sharpless dihydroxylation (80% ee). Therefore, the carbonyl addition toward chiral aldehyde **20** proceeded with excellent diastereoselectivity. The secondary OH functionality was subjected to silyl protection. While most silylation reactions caused complete decomposition of the molecule, only the combination of TBMDSOTf and lutidine gave acceptable results. At −40 °C the side reactions could be suppressed almost completely, and after only 15 min, the desired silyl ether **22** was obtained in good yield. The primary silyl group could selectively be removed in the presence of the secondary one using NH_4F in methanol.[29] This reagent is less reactive than the commonly used Bu_4NF, but it is therefore more selective. Interestingly, no complete conversion was observed, while the primary alcohol **23** was obtained diastereomerically pure. Obviously the silyl ether of the "wrong epoxide" does not undergo cleavage under these reaction conditions. Alcohol **23** was oxidized to the corresponding aldehyde,[30] a rather unstable compound. Workup had to be done at 0 °C and the crude aldehyde was directly converted into methyl ester **24** using *N*-iodosuccinimide (NIS) in MeOH.[31] This ester was saponified directly before the peptide coupling to avoid decomposition of the labile acid.

With these building blocks in hand, we started the synthesis of the linear heptapeptide. Ring closure was planned between the aminohexenoic acid and the *N*-methylated leucine. Therefore, the synthesis started with this amino acid. BEP (2-bromo-1-ethylpyridinium tetrafluoroborate)[32] was used for the coupling with protected valine. Surprisingly, the cleavage of the Cbz-protecting group required a pressure of 20 atm of H_2. HCl (2 equiv) was added to suppress the formation of diketopiperazine. The dipeptide salt was subjected in the next peptide coupling step with activated **13**. Since an excellent yield was obtained, the same protocol was also used in the next coupling step with comparable success. HOBT was used to activate the *N*-methyl-4-hydroxyleucine **9** to avoid epimerization during the coupling step. By this sequence, pentapeptide **29** could be obtained enantiomerically pure in gram scale. For the coupling of the highly sensitive hydroxytryptophan, methyl ester **24** was saponified and the crude product (without purification) was directly coupled using the BEP protocol to give **30** in acceptable yield. The alloc protecting group was removed under Pd catalysis, and the final amino acid **4** was connected. After removing the protecting groups, the deprotected heptapeptide was slowly added to a diluted solution of PyBOP (2 equiv) and base in CH_2Cl_2 (final concentration: 1 mM). Under these conditions, *O*-silylated cyclomarin A (**32**) could be obtained in good yield (Scheme 5). The removal of the two silyl protecting groups was more challenging than expected, since they could not be removed in one step without decomposition. Therefore, we decided to use a two-step protocol where the primary OH group was deprotected first using the NH_4F method. The secondary

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one was removed afterward using exactly 1 equiv of TBAF at 0 °C.

In conclusion, we can show that the unusual amino acids of the cyclomarins can be synthesized in enantiomerically pure form in high yields. With these building blocks in hand, cyclomarin A, the most complex representative of this family of cyclopeptides, could be obtained. Syntheses of the other family members and derivatives thereof for SAR studies are under investigation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03292.

Detailed experimental procedures and copies of NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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